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Letter to the Editor

Sample size in linear regression + of the effect of priming on soil carbon and nitrogen across ecosystems

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One of the findings in the recent study by Mau et al. (2017) in *Applied Soil Ecology* concluded that soil nutrient pools are correlated with altered priming effects across different ecosystems. This finding does not appear to be well-supported by the data in the study due to low sample size and the presence of uncontrolled variables.

Linear regression calculations were provided for the differences in cumulative percent priming (where priming is the difference in the effect of glucose supplementation between ambient and transplanted soils) and changes in carbon and nitrogen pools across four ecosystems (See Fig. 4 in Mau et al., 2017). Although each data point was the average of five measurements, four points (N = 4) for the four ecosystems studied are probably too few for the fit in a multilevel linear regression to be entirely reliable (Maas and Hox, 2005). For example, Moineddin et al. (2007) argued that the minimum group size and group numbers should be 50. The reason for this can be explained if one notes that if one less ecosystem had been studied (N = 3) or one additional ecosystem (N = 5), the slopes that fit to the points would likely have been completely different. In one of the regressions, a significant effect for the positive relationship between total soil carbon and nitrogen and cumulative priming was found exclusively in the pinyon-juniper ecosystem. That is, if this point was removed, the slope of the fit would change dramatically and correlation relationships across ecosystems would disappear. Findings that are dependent on the presence or absence of a single point could naturally have reliability concerns.

Another related concern is that the title and research questions posed in the study assume that any observed carbon and biomass changes would be explained by differences in average annual ambient temperature of the soil samples by virtue of their transplantation at different elevations. In studies that use elevation changes to simulate ambient warming, however, many uncontrolled variables could affect carbon cycling and SOM decomposition. Ambient temperature varies at different elevations, but other differences include incoming nutrients from soils at higher elevations, greater solar radiation at higher elevations, air pressure differences, wind speeds, and others. Any singular or combination of these uncontrolled variables could account for the findings, not just the warmer temperatures associated with lower elevations. Most of these problems could be addressed by reorienting the study from *warming-induced* changes to simply *elevation-induced* changes, which acknowledges implicitly that many variables change with elevation. This would have the beneficial effect of reducing the chance that the readers would misinterpret the nature of the findings. An alternative would be to measure the variables above and correlate them with carbon cycling and SOM decomposition, to see if they have a large, small, or negligible effect.

The strengths of the study are very many, including its nearly decade-long duration, the use of over a dozen of sample time points, the comparison of several different ecosystems to reduce ecosystem dependence of the findings, an excellent control for variable rainfall, and the assessment of priming across soil samples. However, due to a low sample size for the number of ecosystem types samples and the presence of many uncontrolled variables, I suggest that findings on the correlation between priming changes and soil carbon/nitrogen changes be considered tentative and not yet be implemented in soil management and conservation practices.

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